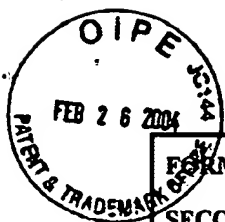


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Application No. <u>09/780,1008</u>	Prepared by <u>DLP</u>	Tracking Number <u>060/3004</u>	
Examiner-GAU <u>Saunders - 1644</u>	Date <u>10/27/04</u>	Week Date <u>9/20/04</u>	
	No. of queries <u>1</u>	<u>IFW (PWTY)</u>	

JACKET			
a. Serial No.	f. Foreign Priority	k. Print Claim(s)	<u>(p) PTO-1449</u>
b. Applicant(s)	g. Disclaimer	l. Print Fig.	q. PTOL-85b
c. Continuing Data	h. Microfiche Appendix	m. Searched Column	r. Abstract
d. PCT	i. Title	n. PTO-270/328	s. Sheets/Figs
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SPECIFICATION	MESSAGE
a. Page Missing	<u>① PTO-1449: Please initial or line through citations on forms dated 2/26/04 (8 sheets)</u>
b. Text Continuity	<u>* Copies provided for reference.</u>
c. Holes through Data	
d. Other Missing Text	<u>② "A6" is being inserted on page 16 of specification, but no "A6" amendment or A-amendment found in file.</u>
e. Illegible Text	<u>please advise/correct.</u>
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<p>CLAIMS</p> <p>a. Claim(s) Missing</p> <p>b. Improper Dependency</p> <p>c. Duplicate Numbers</p> <p>d. Incorrect Numbering</p> <p>e. Index Disagrees</p> <p>f. Punctuation</p> <p>g. Amendments</p> <p>h. Bracketing</p> <p>i. Missing Text</p> <p>j. Duplicate Text</p> <p>k. Other</p>	
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FORM PTO - 1449

SECOND SUPPLEMENTAL INFORMATION
DISCLOSURE STATEMENT

ATTORNEY DOCKET NO.: LEX-011

APPLICANT(S): Gillies *et al.*

SERIAL NO.: 09/780,668

EXAMINER: David A. Saunders

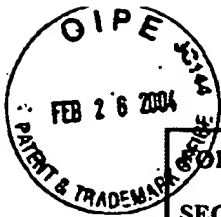
FILING DATE: February 9, 2001 GROUP: 1644

U.S. PATENT DOCUMENTS

EXAM. INIT.		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB CLASS	FILING DATE IF APPROPRIATE
	A60	4,703,008	10/27/87	Lin	435	240.2	11/30/84
	A61	5,457,038	10/10/95	Trinchieri et al.	435	69.52	9/18/90
	A62	5,441,868	8/15/95	Lin	435	69.4	10/23/87
	A63	5,547,933	8/20/96	Lin	514	8	6/7/95
	A64	5,618,698	4/8/97	Lin	435	69.4	6/6/95
	A65	5,650,150	7/22/97	Gillies	424	134.1	7/27/94
	A66	5,688,679	11/18/97	Powell	435	240.2	10/6/93
	A67	5,723,125	3/3/98	Chang et al.	424	134.1	9/25/96
	A68	5,756,349	5/26/98	Lin	435	325	6/6/95
	A69	5,908,626	6/1/99	Chang et al.	424	134.1	12/19/97
	A70	5,955,422	9/21/99	Lin	514	8	8/2/93
	A71	6,100,387	8/8/00	Herrmann et al.	536	23.4	2/28/97
	A72	6,231,536	5/15/01	Lentz	604	5.04	5/21/99
	A73	6,284,536	9/4/01	Morrison et al.	435	328	4/20/99
	A74	6,335,176 B1	1/1/02	Inglese et al.	435	7.72	10/16/98
	A75	6,340,742	1/22/02	Burg et al.	530	351	6/28/00
	A76	6,444,792	9/3/02	Gray et al.	530	387.3	1/8/99
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	A79	6,506,405	1/14/03	Desai et al.	424	450	8/1/00
	A80	6,583,272	6/24/03	Bailon	530	397	6/27/00
	A81	6,586,398	7/1/03	Kinstler et al.	514	12	4/7/00
	A82	6,617,135	9/9/03	Gillies et al.	435	69.7	8/9/00
	A83	2001/0053539 A1	12/20/01	Lauffer et al.	435	69.1	4/6/99

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	A84	2002/0081664 A1	6/27/02	Lo et al.	435	69.5	10/11/01
	A85	2002/0142374 A1	10/3/02	Gallo et al.	435	69.1	8/17/99
	A86	2002/0147311 A1	10/10/02	Gillies et al.	530	387.1	2/9/01
	A87	2002/0192222 A1	12/19/02	Blumberg et al.	424	178.1	8/8/02
	A88	2002/0193570 A1	12/19/02	Gillies et al.	530	351	12/4/01
	A89	2003/0003529 A1	1/2/03	Bayer	435	68.1	7/19/02
	A90	2003/0044423 A1	3/6/03	Gillies et al.	424	192.1	3/7/02
	A91	2003/0049227 A1	3/13/03	Gillies et al.	424	85.1	6/29/01
	A92	2003/0105294 A1	6/5/03	Gillies et al.	530	351	2/24/99
	A93	2003/0012789 A1	1/6/03	Blumberg et al.	424	145.1	8/8/02
	A94	2003/0157054 A1	8/21/03	Gillies et al.	424	85.1	5/3/02
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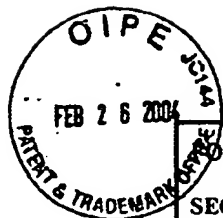
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FOREIGN PATENT DOCUMENTS									
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	B94	WO 97/00319	1/03/97	PCT			6/11/96	N	Y
	B95	WO 99/43713	9/02/99	PCT			2/24/99	N	Y
	B96	WO 00/24893	5/04/00	PCT			10/18/99	N	
	B97	WO 01/36489 A2	5/25/01	PCT			11/03/00	N	Y
	B98	WO 01/58957 A2	8/16/01	PCT			2/09/01	N	Y
	B99	WO 02/02143 A2	1/10/02	PCT			06/29/01	N	Y
	B100	WO 02/066514 A2	8/29/02	PCT			2/18/02	Y	Y
	B101	WO 02/072605 A2	9/19/02	PCT			3/07/02	N	Y
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	B106	WO 03/077834 A2	9/25/03	PCT			7/03/02	N	Y

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EXAM. INIT.	OTHER DOCUMENTS: (Including Author, Title, Date, Relevant Pages, Place of Publication)		
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	C182	Duncan et al., (1988), "The binding site for C1q on IgG," <u>Nature</u> , 332:738-740.	
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EXAM. INIT.	OTHER DOCUMENTS: (Including Author, Title, Date, Relevant Pages, Place of Publication)	
	C186	Fibi et al., (1995), "N- and O-Glycosylation Muteins of Recombinant Human Erythropoietin Secreted From BHK-21 Cells," <u>Blood</u> , 85:1229-1236.
	C187	Frost et al., (1997), "A Phase I/IB Trial of Murine Monoclonal Anti-GD2 Antibody 14.G2a plus Interleukin-2 Children with Refractory Neuroblastoma", <u>Cancer</u> , 80:317-33.
	C188	Gan et al., (1999), "Specific enzyme-linked immunosorbent assays for quantitation of antibody-cytokine fusion proteins," <u>Clin Diagn Lab Immunol</u> , 6(2):236-42.
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	C214	Mueller et al., (1997), "Humanized porcine VCAM-specific monoclonal antibodies with chimeric IgG2/G4 constant regions block human leukocyte binding to porcine endothelial cells," <u>Molecular Immunology</u> , 34(6):441-452.
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	C218	Ngo et al., (1994), "Computational Complexity, Protein Structure Prediction, and the Levinthal Paradox" pp. 433-440 and 492-495.
	C219	Niethammer et al., (2001) "An oral DNA vaccine against human carcinoembryonic antigen (CEA) prevent growth and dissemination of Lewis Lung carcinoma in CEA transgenic mice," <u>Vaccine</u> , 20(3-4):421-9.
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	C222	Pancook et al., (1996), "Eradication of established hepatic human neuroblastoma metastases in mice with severe combined immunodeficiency by antibody-targeted interleukin-2," <u>Cancer Immunol Immunother.</u> , 42(2):88-92.
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	C231	Strom et al., (1996), "Therapeutic Approach to Organ Transplantation", <u>Blackwell Science</u> , Chapter 36, pp. 451-456.
	C232	Syed et al., (1998), "Efficiency of signaling through cytokine receptors depends critically on receptor orientation," <u>Nature</u> , 395:511-516.
	C233	Thommesen et al., (2000), "Lysine 322 in the human IgG3 CH2 domain is crucial for antibody dependent complement activation", <u>Mol. Immunol.</u> , 37(16):995-1004.
	C234	Wells, (1990), "Additivity of Mutational Effect in Proteins," <u>Biochemistry</u> , 29(37):8509-8517.
	C235	Xiang et al., (1998), "Induction of persistent tumor-protective immunity in mice cured of established colon carcinoma metastases," <u>Cancer Res.</u> , 58(17):3918-25.
	C236	Xiang et al., (1999) "T Cell memory against colon carcinoma is long-lived in the absence of antigen," <u>J Immunol.</u> , 163(7):3676-83.
	C237	Xiang et al., (2001), "A dual function DNA vaccine encoding carcinoembryonic antigen and CD40 ligand trimer induces T cell-mediated protective immunity against colon cancer in carcinoembryonic antigen-transgenic mice," <u>J Immunol.</u> , 167(8):4560-5.
	C238	Xiang et al., (2001), "Protective immunity against human carcinoembryonic antigen (CEA) induced by an oral DNA vaccine in CEA-transgenic mice," <u>Clin Cancer Res.</u> , 7(3 Suppl):856s-864s.
	C239	Yu et al., (1998), "Phase I Trial of a Human-Mouse Chimeric Anti-Disialoganglioside Monoclonal Antibody ch14.8 in Patients With Refractory Neuroblastoma and Osteosarcoma", <u>J. Clin. Oncol.</u> , 16:2169-80.
	C240	Zagozdzon et al., (1999), "Potentiation of antitumor effect of IL-12 in combination with paclitaxel in murine melanoma model in vivo," <u>International Journal of Molecular Medicine</u> , 4:645-648.

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EXAMINER	DATE CONSIDERED
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Examples

Example 1. Construction of antibody-IL-2 genes with substitutions of the Lys codon at the fusion junction

5 The amino acid sequence at the junction of the antibody-IL-2 fusion protein is SerProGlyLys-AlaProThr (SEQ ID NO: 1), in which the SerProGlyLys (SEQ ID No. 2) is the normal carboxy terminus of the heavy chain of the antibody, and AlaProThr is the N-terminal sequence of mature IL-2. In order to determine the effect alterations in the region of the fusion junction on the pharmacokinetics of the fusion protein, substitutions or deletion of the residue were made by mutagenesis, as described below:

The expression vector for immunocytokines was described in Gillies *et al.*, (1998) J. Immunol. 160:6195-6203. In the human gamma-1 gene encoding the heavy chain, the XmaI restriction site located 280 bp upstream of the translation stop codon was destroyed by introducing a silent mutation (TCC to TCA). Another silent mutation (TCT to TCC) was introduced to the Ser codon three residues upstream of the C-terminal lysine of the heavy chain to create the sequence TCC CCG GGT AAA (SEQ ID No. 3), which contains a new XmaI site [Lo *et al.*, (1998) Protein Engineering 11:495-500]. The IL-2 cDNA was constructed by chemical synthesis and it contains a new and unique PvuII restriction site [Gillies *et al.*, (1992) Proc. Natl. Acad. Sci. 89:1428-1432]. Both the XmaI and PvuII sites are unique in the expression vector, and they facilitated mutagenesis of the lysine codon which lies at the junction of the CH3 and the IL-2 DNA.

Substitution or deletion of the Lys codon was achieved by replacing the XmaI-PvuII fragment in the immunocytokine expression vector with an oligonucleotide duplex encoding the desired mutation. In this case the variable regions of the heavy and light chains were derived from the humanized KS antibody, which recognized a human antigen called EpCAM (Epithelial cell adhesion molecule). The sequences of the oligonucleotide duplexes used in the present invention are listed below, where the codons in bold encode the desired mutations, and the sequences in italics, CCCGG and CAG are the cohesive end of the XmaI site and the blunt end of the PvuII site, respectively. The